Review of:

Testing the taxonomic validity of Preble's meadow jumping mouse (<u>Zapus hudsonicus preblei</u>).

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I was asked to address five issues in this report. I will address each of them to various degrees based on my understanding of the concepts and analyses employed. I will then provide an overall opinion on the content and context of this study with regard to the relevant conservation issues.

1. <u>Do the data support conclusions that *Z. h. campestris* and *Z. h. preblei* should be synonymized?</u>

I believe that the data support a lack of substantive morphological, ecological, and molecular differentiation between these two subspecies. This is not surprising, and in fact is a very common outcome of molecular analyses of taxonomic subspecies that are in close geographic proximity, are ecologically similar, and appear to have no surmounting biogeographic obstacles to movements across an historical landscape (e.g., as it might have existed during a Late Pleistocene pluvial period). The mtDNA data, specifically, support the contention that populations within the ranges of these two subspecies do not form "reciprocally monophyletic" evolutionary lineages relative to one another, although further sampling of additional specimens and localities, and use of other phylogeographic statistical procedures, might reveal a significantly non-random geographic partitioning of genetic variation into subsets of populations that are largely congruent with a subspecies dichotomy. The latter comment comes from examination of the mtDNA gene phylogeny in the report, in which all four mtDNA haplotypes labeled as "C/P" form a loose, nonsupported by bootstrap replication procedures, aggregrate that might have geographic connections between Colorado and northern populations along major river drainages. If this were true, it might suggest a separate, albeit shallow, geographic history of range connection between certain subsets of populations relative to others within the overall two-subspecies aggregate.

2. Are additional genetics studies required?

Two potential problems are associated with the use of a single genetic marker: it might not reflect "overall" evolutionary affinities between lineages due to sorting or to introgression effects; and it might not be evolving rapidly enough to capture an evolutionary distinction between lineages. The first issue is not likely to be a serious problem in this case. The geographic structure of *campestris* and *preblei* (small, disjunct populations occurring at the edge of the species western distribution) suggests that, while

gene flow between populations may have been frequent under past climatic and habitat conditions, it is not likely to be occurring with high frequency at this time. Perhaps, an original and very distinct "preblei" lineage was swamped by a general dispersal of "campestris" genes into its range, but this seems unlikely to have happened simultaneously across the entire range of preblei given the generally fragmented nature of its populations. The second issue is also not likely to be an issue, because while microsatellites or SNPs might allow one to detect finer-scale population structure than mtDNA, they are not likely to provide a signature of substantial evolutionary subdivision at the level of taxonomic subspecies if the mtDNA did not do so.

3. What is the importance of ecological, behavioral, or physiological differences between subspecies in supporting or refuting synomy?

I have little to say about this issue, in part because I doubt that one would find interesting biological differences between populations representing the different subspecies (they all should occupy a range of rather similar, discrete microhabitats that would select for quite similar phenotypic traits relative to, say *hudsonius* habitats embedded within in a far northern boreal forest), and in part because, without a genetic signature of historical evolutionary separation between lineages (e.g., reciprocal monophyly, or at least significant frequency differences), I would not support recognition of taxonomic distinctness based solely on ecological, behavioral, or physiological traits.

4. What is the likelihood that *preblei* is substantially reproductively isolated from *campestris*?

I see neither a genetic nor a biogeographic reason to predict that populations within these two subspecies are reproductively isolated from one another. Note that reproductive isolation has never been a criterion employed by mammalogists to recognize distinct taxa at the level of subspecies, so the question actually is not relevant to the issue of a substantial and recognizable history of isolation and divergence between populations.

5. Would loss of *preblei* represent a substantial diminution of *campestris* range, biology, or evolutionary legacy?

I suspect that populations of what is now considered *preblei* represent a substantial and important portion of the overall viability of what is now considered *campestris*. My opinion has to do with the fact that both subspecies represent a set of disjunct, peripheral populations at the western edge of the range of the species. Macroecologists identify core vs. peripheral ecological characteristics of species ranges, and these include, for example, a core to peripheral decrease in overall quality and continuity of habitat; and thus a core to peripheral decrease in overall population connectivity, population abundance, and population viability. I suspect that both subspecies represents, in large part, a set of populations that are disconnected from one another, restricted to small, discrete and isolated microhabitats surrounded by decidedly non-*hudsonius* habitats, and thus quite vulnerable to local extinctions without the possibility of rescue via dispersal from surrounding populations. Nevertheless, because of their peripheral isolation to,

perhaps, a subset of what might be considered as ecologically and physiologically extreme environments for the species, these populations could well contain a set of ecological traits that have selective advantage in extreme environments and therefore are unique and interesting (perhaps irreplaceable) within the context of the species as a whole. As such, even if the two subspecies are taxonomically synonymized, I would strongly urge agencies and recovery teams to regard all remaining populations as potentially valuable within a recovery plan.

Final comment

While I see no reason to support an opinion that *preblei* and *campestris* should be retained as separate taxonomic subspecies, I believe that there is still a case to be made for considering the collective set of populations originally represented as separate subspecies as an evolutionary lineage of conservation concern. That this is, perhaps, a highly distinct evolutionary lineage is supported by the very clear and deep "reciprocally monophyletic" separation between *luteus/pallidus* vs. a *campestris/preblei* mtDNA lineages (although, I would have liked to see sequence divergence data in the report, and don't understand why the authors would not want to show results of all analyses rather than just saying (pg. 19) that "Other methods of phylogenetic analysis produced very similar trees."

What this study has done, in my opinion, is not reduced the level of conservation concern for a set of vulnerable populations along the western periphery of the species' distribution, but rather, established that the issue of evolutionary distinction needs to be addressed at a different geographic and sampling scale. If one looks at the proportion of the overall species distribution sampled in this study, it becomes very apparent to me that one cannot really say anything yet about context of the *campestris/preblei* within the phylogeographic structure of the species. Yes, they are quite distinct from luteus/pallidus populations as far south as New Mexico/Arizona and as far east as Kansas/Nebraska, but that still leaves us with at least two alternative hypotheses—either *campestris/preblei* represents its own unique evolutionary lineage (e.g., ESU) within Z. hudsonius; or it represents a western (and / or southern) extension of a lineage that is widespread throughout a larger portion of the species. If I had to make a prediction, after looking at distributional details in the map produced by Dr. Hafner within the context of the overall distribution of the species, I would guess that the mtDNA lineage represented in this study by populations assigned to *luteus* and *pallidus* is the one that represents a subset of a more widely-distributed lineage, and that the *campestris/preblei* lineage really is an evolutionarily distinct subset of the species. This possibility, however, cannot be evaluated without additional population and geographic sampling, although such could be accomplished efficiently by using the same mtDNA genetic marker as used in this report.